REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	1 Jan 98	Final Report 8/1/93 - 7/31/96

4. TITLE AND SUBTITLE
Molecular Approaches to Optical Biosensors

5. FUNDING NUMBERS
N00014-93-1-1245

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Office of Naval Research
800 N. Quicy St.
Arlington, VA 22217-5000

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

19990701 037

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The goal of this proposal was to develop methodologies for the optimization of field-deployable optical biosensors, in general, and, in particular, to optimize a carbonic anhydrase-based fiber optic zinc biosensor. We used both site-directed and random mutagenesis coupled with phage display to prepare and identify carbonic anhydrase (CA) variants that: 1) enhance the kinetics of metal ≥ 1000-fold such that zinc equilibration in the nM range occurs within seconds; 2) alter the zinc affinity by a factor of 10^7-fold (20 fM - 0.2 μM) for use in a biosensor array; and 3) improve the metal detection limit by altering the transduction scheme. Additionally, we have developed phage display technology to screen for CA variants with alter zinc affinity.

14. SUBJECT TERMS
Metals, biosensors, phage display, mutagenesis

15. NUMBER OF PAGES
4

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	UL

FINAL REPORT

Grant#: N00014-93-1-1245

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GRANT TITLE: Molecular Approaches to Optical Biosensors

AWARD PERIOD: 1 August 1993 - 31 July 1996

OBJECTIVE: To develop methodologies for the optimization of field-deployable optical biosensors; to improve the zinc detection limit and speed of equilibration of a carbonic anhydrase-based fiber optic zinc biosensor.

APPROACH: Use both site-directed and random mutagenesis coupled with activity screens to prepare and identify carbonic anhydrase (CA) variants that: 1) increase the kinetics of metal binding; 2) alter the affinity and specificity of the metal site; and 3) improve the metal detection limit by altering the transduction scheme.

ACCOMPLISHMENTS:

Site-directed mutagenesis: To investigate the molecular mechanism for slow zinc equilibration we have prepared CA variants in two amino acids, Gln92 and Glu117, which form hydrogen bonds with His94 and His119. Substitution of Gln92 causes modest increases in both the zinc K_D and the dissociation rate constant. Substitution of Glu117 also causes modest increases (<10-fold) in the zinc K_D; however, the **zinc association and dissociation rate constants increase dramatically** (1000-fold) with little apparent effect on catalytic activity (except for Glu117→Gln) or protein stability. In fact, the Glu117→Gln variant decreases the half-time for equilibration with 0.5 nM zinc from 1 hour to 5 seconds.

We have demonstrated that the histidines that coordinate zinc in CAII can be functionally replaced by asparagine, glutamine, glutamate, aspartate, and cysteine. This causes large (10^5-fold) increases in the zinc dissociation rate constant which are accompanied by large decreases in the binding constants for both zinc. These variants may be particularly useful for detecting nM concentrations of zinc in an equilibrium manner. We have also inserted a fourth proten ligand into the zinc coordination polyhedron of carbonic anhydrase II which **increases the metal affinity 130-fold to 20 fM. To date, we have prepared a collection of CAII variants with zinc affinity varying**

by a factor of 10^7-fold (20 fM - 0.2 μM) for use in a biosensor array.

Additionally, we have supplied our collaborator, Dr. Richard Thompson, CA variants containing one cysteine substituted at a number of positions at different distances from zinc, including Y7C (12Å), N67C (9.6Å), H64C (9Å), V143C (7.1Å) and L198C (7.8Å) CAII. Dr. Thompson's laboratory has labeled this thiol with a fluorophore to detect the binding of a variety of metals by fluorescence energy transfer or fluorescence quenching. These are steps toward the development of CA biosensors that can detect several metals.

Phage display: In order to display CA on the surface of filamentous phage as a functional CA-gene 3 protein (g3p) fusion protein the CA gene has been subcloned into a commercial phagemid vector, pCANTAB, which inserts the CA sequence between the g3p leader sequence and coding region. We successfully displayed active CA on the surface of filamentous phage, developed a sulfonamide affinity selection method capable of enriching CA-phage 1000-fold and used this method to screen for CA variants with altered zinc affinity. We used cassette mutagenesis to prepare a library of CA variants with mutations in three hydrophobic residues, Phe93, Phe95 and Trp97, flanking the histidine residues and used affinity selection to define the variants with the highest zinc affinity. The selected variants that are not wild-type are enriched in the following amino acids: 93-Phe, Ile, Leu, Met; 95-Met, Ile, Leu; and 97-Val, Trp. The zinc affinity of these variants is comparable to wild-type (1-20 nM) but the dissociation rate constant decreases up to 20-fold. The selected variants differ when cobalt is substituted for zinc suggesting that we can **alter metal ion specificity** as well.

SIGNIFICANCE: We have prepared CAII variants which significantly affect both the half-time for equilibration and the zinc dissociation constants. These will allow the zinc biosensor to be utilized in an array fashion for continuous monitoring. We have prepared single cysteine mutants of CA that can be specifically labeled with fluorophores for reagentless sensing of metal ions. Furthermore, we have set up a method using phage display to screen for useful CA variants.

PATENT INFORMATION: None

AWARD INFORMATION: None

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